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# RP-HPLC Method for Simultaneous Quantification and Validation of Sofosbuvir and Velpatasvir in Bulk Drugs and Formulations

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**Abstract –** Pharmaceutical analysis is crucial for both quality assurance and quality control of bulk drugs and pharmaceutical formulations. Analytical method development has become a fundamental activity in pharmaceutical analysis by using advanced analytical techniques like GC, HPLC, IC, LCMS, GCMS etc. Analytical techniques are developed and validated for a range of substances including active pharmaceutical ingredients (APIs), excipients, drug products, degradation products, related substances and residual solvents. These validated methods have become essential requirements for regulatory organizations. Regulatory authorities are increasingly emphasizing the importance of robust analytical methods in the manufacturing process. To gain drug approval, applicants must demonstrate comprehensive control over the drug development process using validated analytical methods**. I. Introduction**

Sofosbuvir and Velpatasvir combination is used in the treatment of chronic hepatitis C virus (HCV) infection.

Sofosbuvir is Isopropyl (2S)-2-[[[(2R, 3R, 4R, 5R)-5-(2, 4-dioxopyrimidin-1- yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl] methoxy-phenoxy-phosphoryl] amino] propanoate which was shown in figure. Sofosbuvir inhibits the hepatitis C Nonstructural protein 5B (NS5B) is a viral protein found in the hepatitis C virus (HCV). It is an RNA-dependent RNA polymerase, having the key function of replicating HCV's viral RNA by using the viral positive RNA strand as a

template to catalyze the polymerization of ribonucleoside triphosphates (rNTP) during RNA replication[1-4].

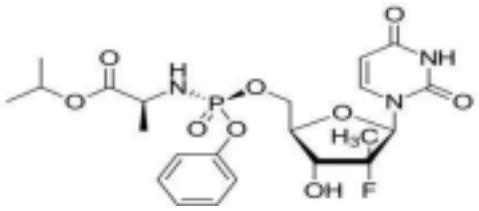


**Fig. No. Structure of Sofosbuvir**

Velpatasvir is Methyl{(2S)-1-[(2S,5S)-2-(9-{2-[(2S,4S)-1-{(2R)-2-[(methoxy carbonyl) amino]-2-phenyl acetyl}-4-(methoxy methyl)-2-pyrrolidinyl]-1H-imidazol-4-yl}-1,11-dihydroisochrome no [4',3':6,7] naphtho[1,2-d]imidazol-2-yl)-5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl} carbamate which was shown in figure.

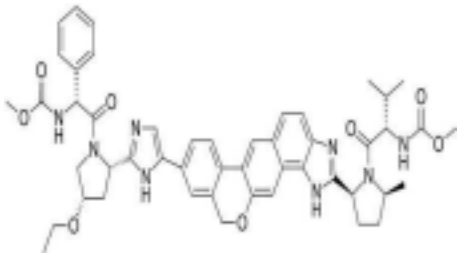


**Fig. No. Structure of Velpatasvir**

Velpatasvir is an inhibitor of Nonstructural protein 5A (NS5A) and a component of the transporter proteins P-glycoprotein (Pgp) and ATP-binding cassette super-family G member 2[5]. Literature review reveals that very few methods were reported for the quantification of Sofosbuvir and Velpatasvir individually and combined with other drugs. However, previously few methods were reported either single or combination with other drugs for the quantification of Sofosbuvir and Velpatasvir by HPLC [6-12,] HPTLC [13,14,] LCMS[15-17] and UV Spectrophotometric [18,19] methods.

Hence, in the present study an attempt has been made by the author to develop a rapid and reliable HPLC method for determination of Sofosbuvir and Velpatasvir. The HPLC technique was successfully employed to provide satisfactory, sensitivity and selectivity in a desirable time of chromatographic run.

## II. Materials and Methods

**Instruments required:**

A RP-HPLC method was performed on a Waters Alliance 2695 HPLC system equipped with 2998 Photo Diode Array (PDA) detector, autosampler and Empower 2 software for processing and data

collecting. BDS C8 (4.6 x 50 mm, 5 µm) was used as stationary phase. An ultrasonic bath sonicator (Frontline FS 4, Mumbai, India), Denver electronic balance and Whatman filter paper No. 41 is used in the study.

**Reagents used:**

Sofosbuvir and Velpatasvir were procured from Hetero Drugs Limited, Hyderabad, India. Acetonitrile of HPLC grade were procured from SD Fine Chemicals, India. Water was obtained from Milli-Q. Ortho-phosphoric acid and Potassium dihydrogen ortho-phosphate of HPLC grade was procured from Rankem Ltd., India.

**Preparation of mobile phase:**

1.36g of potassium dihydrogen phosphate was accurately weighed and transferred to 1000 ml reagent bottle containing 900 ml of Milli Q grade water. The contents were shaken and volume made with water. The pH of the buffer was adjusted to 3.5 with 0.1%OPA. The buffer was then filtered through 0.45µm pore size filter paper before use and mixed with HPLC grade Acetonitrile with 50:50, v/v.

**Diluent:**

Based upon the solubility of the drug, diluents were selected and diluted with Acetonitrile and buffer with 50:50 ratio.

**Preparation of Standard stock solutions**: Accurately weighed 40 mg of Sofosbuvir and 5 mg of Velpatasvir transferred to 50 ml volumetric flask. 10 ml of Diluent was added to flask and sonicated for 10 mins. Flask was made up with the diluent to get the concentration 800 µg/ml of Sofosbuvir and 100 µg/ml Velpatasvir.

**Preparation of Standard working solutions:** 1.0 ml from each stock solution was pipette out and taken into a 10 ml volumetric flask and made up with the diluent to get the concentration 80µg/ml of Sofosbuvir and 10µg/ml of Velpatasvir.

**Preparation of Sample Stock Solution:** Weighed about minimum 5 tablets and crushed into a fine powder and calculate the average weight of each tablet then the weight equivalent to 1 tablet wastaken and transferred into a 50 ml volumetric flask, to this added 10 ml of diluent and sonicated for 25 min, further the volume made up with diluent and filtered using a 0.45 µm nylon filter and the resulting solution was centrifuged at 3000 rpm for 5 minutes. And after the dilution was made up with equivalent to get the concentration 800 µg/ml of Sofosbuvir and 100 µg/ml of Velpatasvir.

**Preparation of Sample working solutions**: From the filtered solution 1 ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluents to get the concentration 80µg/ml of Sofosbuvir and 10µg/ml of Velpatasvir.

**Standard and sample solution for assay studies:**

An aliquot of 10 µL of standard and sample solution contains 80µg/ml of Sofosbuvir and 10µg/ml of Velpatasvir were injected six times into the chromatographic system and peak area for Sofosbuvir and Velpatasvir were measured and assay % was calculated by comparing the peak area of standard and sample chromatogram was shown in Table.

**Assay:**

Assay was performed with the above solution. The average % Assay for Sofosbuvir and Velpatasvir obtained was 99.84% and 99.87% respectively.

**Table: Assay Data of Sofosbuvir**

| S.no | Standard Area | Sample area | % Assay |
|---|---|---|---|
| 1 | 2089767 | 2090768 | 100.26 |
| 2 | 2097171 | 2079433 | 99.72 |
| 3 | 2084873 | 2090725 | 100.26 |
| 4 | 2054911 | 2087749 | 100.12 |
| 5 | 2073048 | 2061219 | 98.84 |
| 6 | 2099592 | 2082340 | 99.86 |
| **Avg** | 2083227 | 2082039 | 99.84 |
| **SD** | 16811.5 | 11176.8 | 0.54 |
| **%RSD** | 0.8 | 0.5 | 0.54 |

**Table: Assay Data of Velpatasvir**

| S.no | Standard Area | Sample area | % Assay |
|---|---|---|---|
| 1 | 587753 | 590756 | 99.83 |
| 2 | 583205 | 591666 | 99.99 |
| 3 | 592039 | 594272 | 100.43 |

| | | | |
|---|---|---|---|
| 4 | 597173 | 589801 | 99.67 |
| 5 | 591419 | 588396 | 99.43 |
| 6 | 595330 | 590832 | 99.85 |
| **Avg** | 394069 | 590954 | 99.87 |
| **SD** | 5084.8 | 1971.9 | 0.33 |
| **%RSD** | 0.9 | 0.3 | 0.33 |

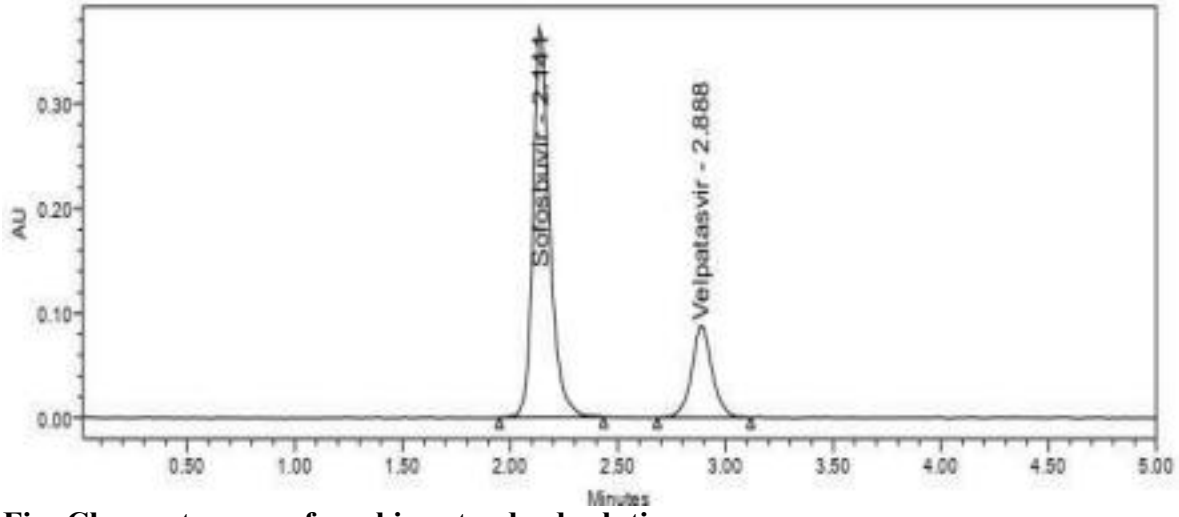



**Fig: Chromatogram of working standard solution**

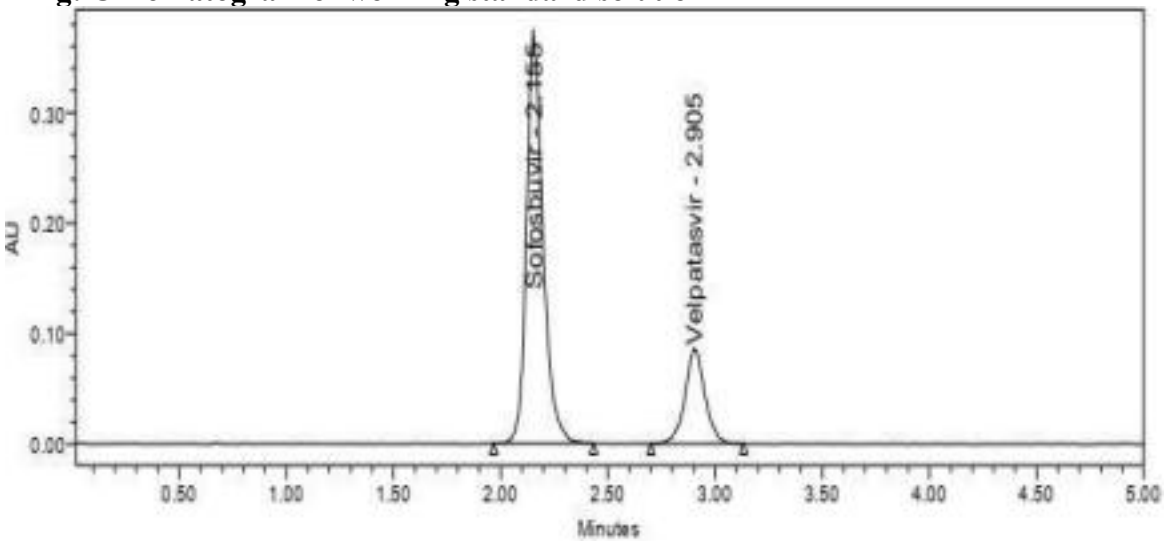



**Fig: Chromatogram of working sample solution**

**Table: Results for assay studies**

| Drug | Label claim (mg/tablet) | Amount found* | Label claim % | RSD % |
|---|---|---|---|---|

| | | (mg/tablet) | | |
|---|---|---|---|---|
| Sofosbuvir | 400 | 399.36 | 99.84 | 0.54 |
| Velpatasvir | 100 | 99.87 | 99.87 | 0.33 |

* Mean of six determinations

## III. METHOD VALIDATION

**System suitability parameters**:

The system suitability parameters were determined by preparing standard solutions of Sofosbuvir and Velpatasvir (80µg/ml of Sofosbuvir and 10µg/ml of Velpatasvir) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

**Specificity:**

Checking of the interference in the optimized method, it was found that no interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

**Precision**:
**Preparation of Sample Stock Solution:** Weighed about minimum 10 tablets and crushed into a fine powder with mortar and pestle and calculate the average weight of each tablet then the weight equivalent to 1 tablet was taken and transferred into a 50 ml volumetric flask, to this added 10 ml of diluent and sonicated for 25 min, further the volume made up with diluent and filtered using a 0.45 µm nylon filter and the resulting solution was centrifuged at 3000 rpm for 5 minutes. And after the dilution was made up with equivalent to get the concentration 800 µg/ml of Sofosbuvir and 100 µg/ml of Velpatasvir.

**Preparation of Sample working solutions**: From the filtered solution 1 ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluents to get the concentration 80µg/ml of Sofosbuvir and 10µg/ml of Velpatasvir. The precision was determined by

preparing sample solutions of 80µg/ml of Sofosbuvir and 10µg/ml of Velpatasvir and the solutions were injected six times, the % RSD for the area of six standard injections results should not be more than 2%.

**Linearity**:

**Preparation of Standard stock solutions**: Accurately weighed 40 mg of Sofosbuvir and 5 mg of Velpatasvir transferred to 50 ml volumetric flask. 10 ml of Diluent was added to flask and sonicated for 10 mins. Flask was made up with the diluent to get the concentration 800 µg/ml of Sofosbuvir and 100 µg/ml Velpatasvir.

**25% Standard solution**: 0.25ml each from three standard stock solutions was pipette out and made up to 10ml to get the concentration 20µg/ml of Sofosbuvir and 2.5µg/ml of Velpatasvir.

**50% Standard solution**: 0.5ml each from three standard stock solutions was pipette out and made up to 10ml to get the concentration 40µg/ml of Sofosbuvir and 5µg/ml of Velpatasvir.

**75% Standard solution**: 0.75ml each from three standard stock solutions was pipette out and made up to 10ml to get the concentration 60µg/ml of Sofosbuvir and 7.5µg/ml of Velpatasvir.

**100% Standard solution**: 1.0 ml each from three standard stock solutions was pipette out and made up to 10 ml to get the concentration 80 µg/ml of Sofosbuvir and 10 µg/ml of Velpatasvir.

**125% Standard solution**: 1.25 ml each from three standard stock solutions was pipette out and made up to 10 ml to get the concentration 100 µg/ml of Sofosbuvir and 12.5 µg/ml of Velpatasvir.

**150% Standard solution**: 1.50 ml each from three standard stock solutions was pipette out and made up to 10 ml to get the concentration 120 µg/ml of Sofosbuvir and 15 µg/ml of Velpatasvir.

**Accuracy**:

**Preparation of Standard stock solutions**: Accurately weighed 40mg of Sofosbuvir and 5mg of Velpatasvir transferred to 50ml volumetric flask. 10ml of Diluent was added to

flask and sonicated for 10mins. Flask was made up with the diluent to get the concentration 800 µg/ml of Sofosbuvir and 100 µg/ml Velpatasvir.

**Preparation of Sample stock solutions**: 10 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was taken and finely powdered with mortar and pestle and transferred into a 50 mL volumetric flask, 10mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered and the resulting solution was centrifuged at 3000 rpm for 5 min and after suitable dilution the sample solution was then filtered using 0.45-µm nylon filter to get the concentration 800µg/ml of Sofosbuvir and 100µg/ml Velpatasvir.

**Preparation of 50% Spiked Solution**: 0.500 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipetted out and made up to the mark with diluent.

**Preparation of 100% Spiked Solution**: 1.00 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipetted out and made up to the mark with diluent.

**Preparation of 150% Spiked Solution**: 1.500 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out and made up to the mark with diluent.

**Acceptance Criteria**:

The % Recovery for each level should be between 98.0 to 102.0.

**Robustness:** Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there was no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9 ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

**LOD Sample Preparation**: 0.25 ml of standard stock solutions was pipette out and transferred to 10 ml volumetric flask and made up with diluents from the above solutions

0.1ml of Sofosbuvir and Velpatasvir solutions respectively were transferred to 10 ml volumetric flasks and made up with the same diluents.

**LOQ Sample Preparation**: 0.25 ml of standard stock solutions was pipette out and transferred to 10 ml volumetric flask and made up with diluents from the above solutions 0.1 ml of Sofosbuvir and Velpatasvir solutions respectively were transferred to 10 ml volumetric flasks and made up with the same diluents.

**Degradation studies**:

**Oxidation:**

To 1.0 ml of stock solutions of Sofosbuvir and Velpatasvir, 1.0 ml of 20% hydrogen peroxide ($H_2O_2$) was added separately. The solutions were kept for 30 min at $60^0$C. For HPLC study, the resultant solution was diluted to obtain 80µg/ml of Sofosbuvir and 10µg/ml of Velpatasvir and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Acid Degradation Studies**:

To 1.0 ml of stock solution Sofosbuvir and Velpatasvir, 1.0 ml of 2N Hydrochloric acid was added and refluxed for 30mins at $60^0$C.The resultant solution was diluted to obtain 80 µg/ml of Sofosbuvir and 10 µg/ml of Velpatasvir and 10µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Alkali Degradation Studies**:

To 1.0 ml of stock solution Sofosbuvir and Velpatasvir, 1.0 ml of 2N sodium hydroxide was added and refluxed for 30mins at $60^0$C. The resultant solution was diluted to obtain 80 µg/ml of Sofosbuvir and 10µg/ml of Velpatasvir and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Thermal Degradation Studies**:

The standard drug solution of Sofosbuvir and Velpatasvir was placed in oven at $105^0$C for 1h to study dry heat degradation. For HPLC study, the resultant solution was diluted obtain 80 µg/ml of Sofosbuvir and 10 µg/ml of Velpatasvir and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

**Photo Stability studies**:

The photochemical stability of the drug was also studied by exposing the Sofosbuvir and Velpatasvir with concentration 80 µg/ml of Sofosbuvir and 10 µg/ml of Velpatasvir solution

to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/$m^2$ in photo stability chamber and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Neutral Degradation Studies**:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to obtain 80 µg/ml of Sofosbuvir and 10 µg/ml of Velpatasvir and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

**Results and discussion:**

Method development: Method development was done by changing various columns, mobile phase ratios, buffers and its pH etc.

**Optimized method:**
**Chromatographic conditions:**

**Mobile phase :** 50% 0.1N KH2PO4 buffer: 50% Acetonitrile **Flow rate :** 1.0 ml/min

**Column :** BDS C8 (4.6 x 50mm, 5µm)

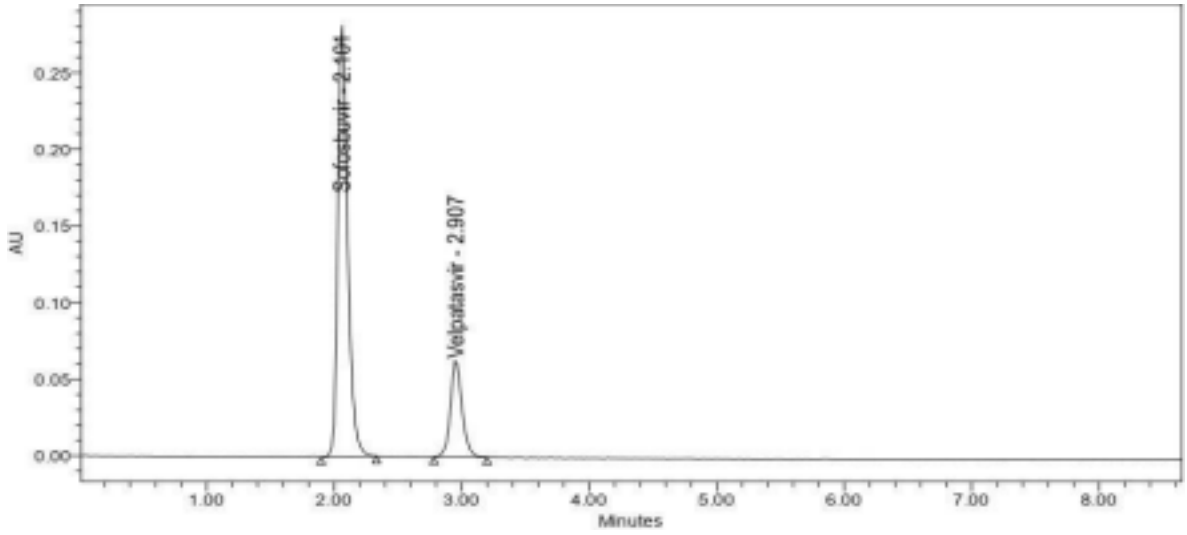
**Detector Wavelength :** 288.0nm

**Column temperature :** 30°C

**Injection volume :** 10µL

**Run time :** 9 min

**Diluent :** Water and Acetonitrile in the ratio 50:50 **Results :** Both peaks have good resolution, tailing factor, theoretical plate count and resolution.




**Optimized Chromatogram**

**Observation:** Sofosbuvir and Velpatasvir were eluted at 2.101min and 2.907min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

## METHOD VALIDATION

**System Suitability**:

**Table: System suitability parameters for Sofosbuvir and Velpatasvir**

| Sl no | Sofosbuvir | | | Velpatasvir | | | |
|---|---|---|---|---|---|---|---|
| **Inj** | **RT(min)** | **TP** | **Tailing** | **RT(min)** | **TP** | **Tailing** | **Resolution** |
| 1 | 2.136 | 3967 | 1.27 | 2.871 | 4916 | 1.05 | 4.7 |
| 2 | 2.138 | 3902 | 1.27 | 2.884 | 4927 | 1.11 | 4.9 |
| 3 | 2.141 | 4049 | 1.28 | 2.887 | 4989 | 1.04 | 4.8 |
| 4 | 2.141 | 4031 | 1.28 | 2.888 | 4844 | 1.05 | 4.7 |
| 5 | 2.152 | 4154 | 1.28 | 2.897 | 5099 | 1.06 | 4.9 |
| 6 | 2.156 | 4146 | 1.30 | 2.907 | 5166 | 1.07 | 4.8 |

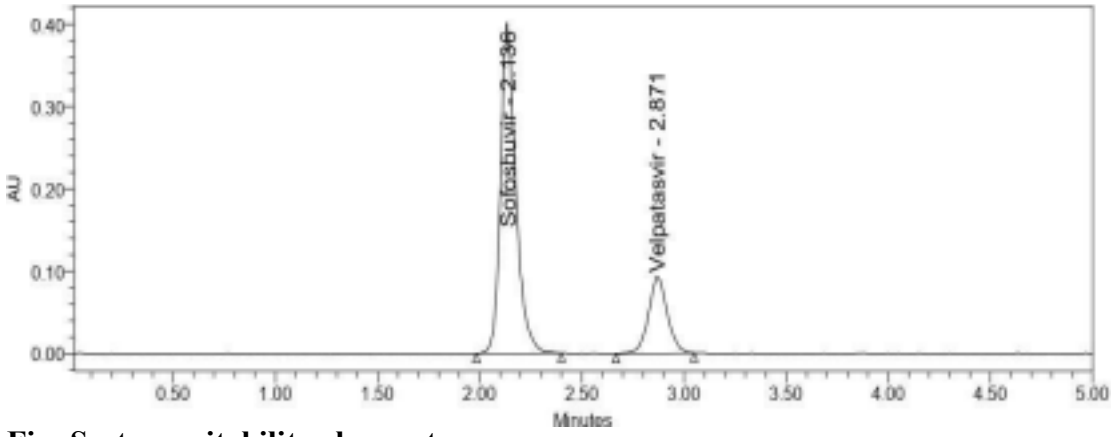



**Fig: System suitability chromatogram**

**Discussion**: Plate count, tailing factor, resolution of Sofosbuvir and Velpatasvir was according to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

**Specificity:**

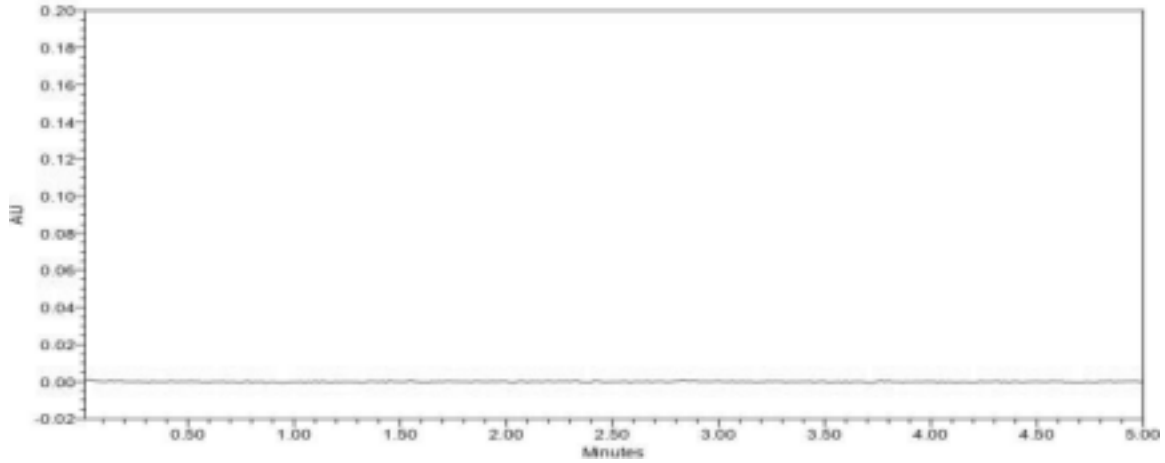



**Fig: Blank chromatogram**

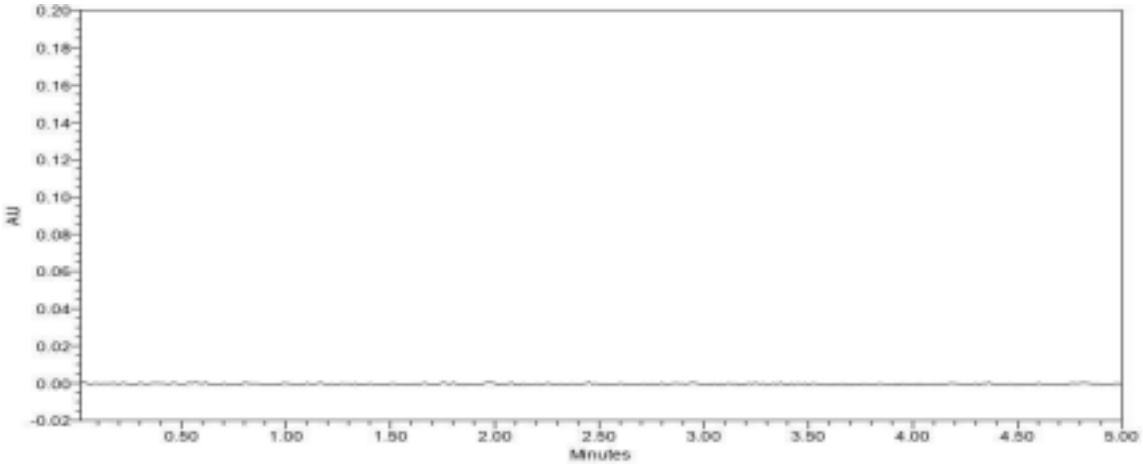


**Fig: Placebo chromatogram**

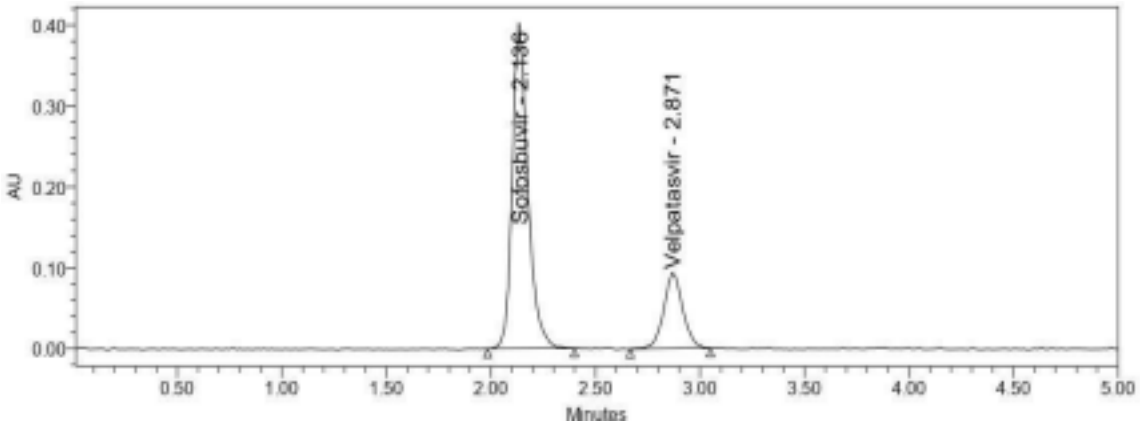



**Fig: Optimized chromatogram**

**Discussion:** Retention times of Sofosbuvir and Velpatasvir were 2.136min and 2.871min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

**Linearity**:

**Table: Linearity table for Sofosbuvir and Velpatasvir**

| **Sofosbuvir** | | **Velpatasvir** | |
|---|---|---|---|
| **Conc (µg/mL)** | **Peak area** | **Conc (µg/mL)** | **Peak area** |
| 20 | 518858 | 2.5 | 151547 |
| 40 | 1047581 | 5 | 297597 |
| 60 | 1502795 | 7.5 | 447465 |
| 80 | 2085606 | 10 | 592112 |
| 100 | 2542313 | 12.5 | 751020 |
| 120 | 3078920 | 15 | 892625 |

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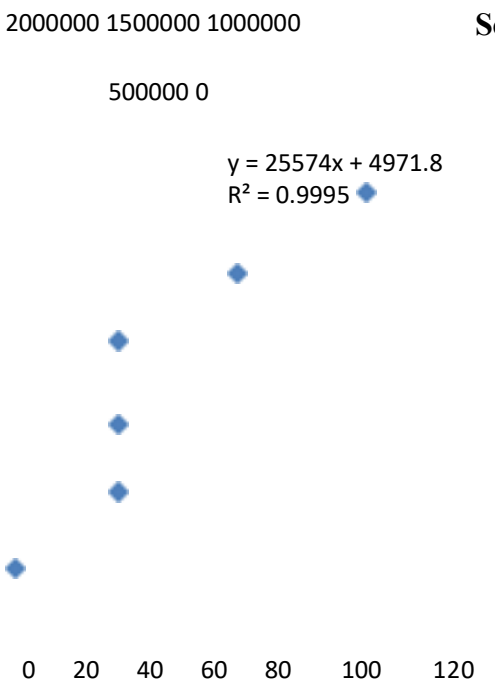


**Sofosbuvir**

140 **Fig: Calibration curve of**


**Fig: Calibration curve of Velpatasvir**

**Discussion:**

Six linear concentrations of Sofosbuvir (20-120µg/ml) and Velpatasvir (2.5-15µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Sofosbuvir was y = 25574x + 4971 and Velpatasvir was y = 74488x + 551.9. Correlation coefficient obtained was 0.999 for the two drugs.

**Precision**:

**System Precision**:

**Table: System precision table of Sofosbuvir and Velpatasvir**

| S. No | Concentration (µg/ml) | Area of Sofosbuvir | Concentration (µg/ml) | Area of Velpatasvir |
|---|---|---|---|---|
| 1. | 80 | 2089767 | 10 | 587753 |
| 2. | 80 | 2097171 | 10 | 583205 |
| 3. | 80 | 2084873 | 10 | 592039 |
| 4. | 80 | 2054911 | 10 | 597173 |
| 5. | 80 | 2073048 | 10 | 591419 |
| 6. | 80 | 2099592 | 10 | 595330 |
| Mean | | 2083227 | Mean | 591153 |
| S.D | | 16811.5 | S.D | 5084.8 |
| %RSD | | 0.8 | %RSD | 0.9 |

**Method Precision:**

**Table: Repeatability table of Sofosbuvir and Velpatasvir**

| S. No | Concentration (µg/ml) | Area of Sofosbuvir | Concentration (µg/ml) | Area of Velpatasvir |
|---|---|---|---|---|
| 1. | 80 | 2090768 | 10 | 590756 |
| 2. | 80 | 2079433 | 10 | 591666 |
| 3. | 80 | 2090725 | 10 | 594272 |
| 4. | 80 | 2087749 | 10 | 589801 |
| 5. | 80 | 2061219 | 10 | 588396 |
| 6. | 80 | 2082340 | 10 | 590832 |
| Mean | | 2082039 | Mean | 590954 |
| S.D | | 11176.8 | S.D | 1971.9 |
| %RSD | | 0.5 | %RSD | 0.3 |

**Discussion:** Multiple sampling from a sample stock solution was done and six working sample

solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.5% and 0.3% respectively for Sofosbuvir and Velpatasvir. As the limit of Precision was less than "2" the system precision was passed in this method.

**Intermediate precision**:

**Table: Intermediate precision table of Sofosbuvir and Velpatasvir**

| S. No | Concentration (µg/ml) | Area of Sofosbuvir | Concentration (µg/ml) | Area of Velpatasvir |
|---|---|---|---|---|
| 1. | 80 | 2040768 | 10 | 584756 |
| 2. | 80 | 2079433 | 10 | 581666 |
| 3. | 80 | 2051725 | 10 | 584272 |
| 4. | 80 | 2037749 | 10 | 583633 |
| 5. | 80 | 2061219 | 10 | 588396 |
| 6. | 80 | 2079307 | 10 | 590832 |
| Mean | | 2058367 | Mean | 585593 |
| S.D | | 18272.0 | S.D | 3376.8 |
| %RSD | | 0.9 | %RSD | 0.6 |

**Discussion:** Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.9% and 0.6% respectively for Sofosbuvir and Velpatasvir. As the limit of Precision was less than "2" the system precision was passed in this method.

**Accuracy**:

**Table: Accuracy table of Sofosbuvir**

| **% Level** | **Amount Spiked (µg/mL)** | **Amount recovered (µg/mL)** | **% Recovery** | **Mean %Recovery** |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| **50%** | 40 | 40.13 | 100.31 | 100.11% |
| | 40 | 39.83 | 99.56 | |
| | 40 | 39.82 | 99.56 | |
| **100%** | 80 | 80.44 | 100.55 | |
| | 80 | 79.76 | 99.70 | |
| | 80 | 79.99 | 99.99 | |
| **150%** | 120 | 122.77 | 102.30 | |
| | 120 | 119.29 | 99.40 | |
| | 120 | 119.51 | 99.59 | |

**Table: Accuracy table of Velpatasvir**

| **% Level** | **Amount Spiked (µg/mL)** | **Amount recovered (µg/mL)** | **% Recovery** | **Mean %Recovery** |
|---|---|---|---|---|
| **50%** | 5 | 4.97 | 99.37 | 99.31% |
| | 5 | 4.93 | 98.56 | |
| | 5 | 4.97 | 99.50 | |
| **100%** | 10 | 9.94 | 99.39 | |
| | 10 | 9.86 | 98.60 | |
| | 10 | 9.96 | 99.64 | |
| **150%** | 15 | 14.89 | 99.27 | |
| | 15 | 14.98 | 99.88 | |
| | 15 | 14.94 | 99.58 | |

**Discussion:** Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean % Recovery was obtained as 100.11% and 99.31% for Sofosbuvir and Velpatasvir respectively.

**Fig No. Accuracy 50% Chromatogram of Sofosbuvir and Velpatasvir**

**Fig No. Accuracy 100% Chromatogram of Sofosbuvir and Velpatasvir**

**Fig No. Accuracy 150% Chromatogram of Sofosbuvir and Velpatasvir Sensitivity**:

**Table: Sensitivity table of Sofosbuvir and Velpatasvir**

| Drugs | LOD(µg/ml) | LOQ(µg/ml) |
|---|---|---|
| **Sofosbuvir** | 0.13 | 0.40 |
| **Velpatasvir** | 0.01 | 0.04 |

**Fig. No. LOD Chromatogram of Standard**

**Fig.No. LOQ Chromatogram of Standard**

**Robustness**:

**Table: Robustness data for Sofosbuvir and Velpatasvir**

| S.no | Condition | %RSD of Sofosbuvir | %RSD of Velpatasvir |
|---|---|---|---|
| 1 | Flow rate (-) 0.9ml/min | 0.4 | 0.2 |
| 2 | Flow rate (+) 1.1ml/min | 0.4 | 0.6 |

| | | | |
|---|---|---|---|
| 3 | Mobile phase (-) 35B:65A | 0.6 | 0.1 |
| 4 | Mobile phase (+) 45B:55A | 0.8 | 0.4 |
| 5 | Temperature (-) 25°C | 0.4 | 0.1 |
| 6 | Temperature (+) 35°C | 0.3 | 0.5 |

**Discussion:** Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (35B:65A), mobile phase plus (45B:55A), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

**Fig No. Flow minus Chromatogram of Sofosbuvir and Velpatasvir**

**Fig No. Flow plus Chromatogram of Sofosbuvir and Velpatasvir**

**Fig No. Mobile phase minus Chromatogram of Sofosbuvir and Velpatasvir**

**Fig No. Mobile phase Plus Chromatogram of Sofosbuvir and Velpatasvir**

**Fig No. Temperature minus Chromatogram of Sofosbuvir and Velpatasvir**

**Fig No. Temperature plus Chromatogram of Sofosbuvir and Velpatasvir**

**DEGRADATION:**

**Degradation Studies:** Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

**Table: Degradation Data of Sofosbuvir**

| S.NO | Degradation Condition | % Drug Undegraded | % Drug Degraded |
|---|---|---|---|
| 1 | Acid | 92.72 | 7.28 |
| 2 | Alkali | 95.54 | 4.46 |
| 3 | Oxidation | 96.66 | 3.34 |
| 4 | Thermal | 97.19 | 2.81 |
| 5 | UV | 98.38 | 1.62 |
| 6 | Water | 98.38 | 1.62 |

**Table: Degradation Data of Velpatasvir**

| S.NO | Degradation Condition | % Drug Undegraded | % Drug Degraded |
|---|---|---|---|
| 1 | Acid | 94.74 | 5.26 |
| 2 | Alkali | 95.00 | 5.0 |
| 3 | Oxidation | 96.29 | 3.71 |
| 4 | Thermal | 97.30 | 2.7 |

| | | | |
|---|---|---|---|
| 5 | UV | 98.43 | 1.57 |
| 6 | Water | 99.57 | 0.43 |

**Fig. No. Acid degradation chromatogram of Sofosbuvir and Velpatasvir**

**Fig. No. Base degradation chromatogram of Sofosbuvir and Velpatasvir**

**Fig. No. Peroxide degradation chromatogram of Sofosbuvir and Velpatasvir**

**Fig. No. Thermal degradation chromatogram of Sofosbuvir and Velpatasvir**

**Fig.No. Photo degradation chromatogram of Sofosbuvir and Velpatasvir**

**Fig.No. Water degradation chromatogram of Sofosbuvir and Velpatasvir**

## IV. Summary and conclusion

A simple, accurate, precise method was developed for the simultaneous estimation of the Sofosbuvir and Velpatasvir in tablet dosage form. Retention times of Sofosbuvir and Velpatasvir were found to be 2.136 min and 2.871 min. % RSD of system precision for Sofosbuvir and Velpatasvir were and found to be 0.8 and 0.9 respectively. % RSD of method precision for Sofosbuvir and Velpatasvir were and found to be 0.5 and 0.3 respectively. % Recovery was obtained as 100.11% and 99.31% for Sofosbuvir and Velpatasvir respectively. LOD values are obtained from regression equations of Sofosbuvir and Velpatasvir were 0.13 µg/ml and 0.01 µg/ml and LOQ values are obtained from regression equations of Sofosbuvir and Velpatasvir were 0.40 µg/ml and 0.04 µg/ml respectively. Regression equation of Sofosbuvir was y = 25574x + 4971 and Velpatasvir was y = 74488x + 551.9. Retention times are decreased. So the method developed was simple and economical that can be adopted in regular quality control test in Industries.

## V. References


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